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A BF SUBTYPE "Fb1" IS A MARKER GENE OF SOME MONGOLOID POPULATIONS

Koichi SUZUKI, Shinya MUTOH, G. CEY-BERT, Shigenori ITO, and Hideo MATSUMOTO

Department of Legal Medicine, Osaka Medical College, 2–7 Daigakumachi, Takatsuki, Osaka 569, Japan

Summary A factor B subtype, BF*Fb1, was first detected in Japanese by using isoelectric focusing or agarose gel electrophoresis in Tris/glycine/Veronal buffer. Our previous studies suggested that BF*Fb1 may be characteristic of some of Mongoloid populations. To investigate further distribution of BF*Fb1, samples randomly collected from Japanese in Yonaguni island of Japan and Cambodian were tested. BF*Fb1 was not observed in a Cambodian population whereas in a Japanese population of Yonaguni island, BF*Fb1 occurred at a frequency five times as high as those in main islands of Japan. In paternity cases, a Korean family with three offsprings was shown to transmit BF*Fb1 from the accused man to one of the offsprings. These data strongly indicate that BF*Fb1 is a marker gene for some of Mongoloid populations.

Key Words factor B (BF), Mongoloid, polymorphism

INTRODUCTION

Factor B (BF) is a single-chain glycoprotein which establish its initial contact with C3b through its N-terminal fragment, Ba, during formation of the C3 convertase in alternative pathway for complement activation (for review see Müller-Eberhard, 1988). The genetic locus of BF is structurally linked to the major histocompatibility complex (MHC) with other complement loci coding for C2, C4A, and C4B and two loci for 21-hydroxylase A (21OHA) and B (21OHB) (Carroll *et al.*, 1984, 1985; White *et al.*, 1985). Its genetic polymorphism has been extensively investigated on various populations since the first report of Alper *et al.* (1972) by using immunofixation agarose gel electrophoresis.

Isoelectric focusing (IEF) technique has provided further possibility to subdivide a single allotype determined by conventional electrophoresis into two subtypes and has also contributed to BF typing (for review see Geserick and Patzelt,

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1988). Four sets of subtype have been recognized for each of the two common alleles, BF*F and BF*S, as follows: FA(=F′, Fb) and FB(=F″, Fa) (Teng and Tan, 1982; Geserick *et al.*, 1983; Abbal *et al.*, 1985; Nagai *et al.*, 1986; Segurado and Arnaiz-Villena, 1989), SA and SB (David *et al.*, 1983), Sb (Weidinger *et al.*, 1984), and Fb1 (Nakamura *et al.*, 1987; Suzuki *et al.*, 1987a, b; FB in Nishimukai *et al.*, 1988). It is reported that the SA-SB subtype set may be misinterpretation resulting from ambiguous banding patterns due to spontaneous BF conversion during sample collection and/or sample storage (Segurado and Arnaiz-Villena, 1989). We have reported the Fb1 subtype by using agarose gel electrophoresis and have shown through conversion analysis that the subtypic polymorphism resides in the Ba fragment (Suzuki *et al.*, 1987a). The Fb1 allele occurs at a polymorphic frequency exclusively in Japanese and Chinese among Asian populations thus far studied, and shows a firm association with C2*C, C4A*3, and C4B*2, thus forming a complotype "Fb1C32" in Japanese (Suzuki *et al.*, 1987a). In this paper, we present further data on BF polymorphism in two Mongoloid populations and discuss the distribution of the major two alleles and the BF*Fb1 allele.

MATERIALS AND METHODS

Samples. EDTA-plasma samples were collected from 99 randomly selected unrelated individuals living in Yonaguni island and sera from 177 Cambodians. Blood samples were also obtained from a Korean family living in Japan tested for paternity. Yonaguni island lies in the most west end of the Yaeyama islands belonging to Okinawa prefecture and lies off the east coast of Taiwan at a distance of 74 km.

Agarose gel electrophoresis (AGE). 0.8% agarose gel was melted with threefold diluted Tris/glycine/Veronal buffer (TGVB, 186 mM Tris/530 mM glycine/ 31 mM disodium barbiturate/ 5.6 mM barbital pH 8.8 described by O'Neill *et al.*, 1978) containing 5 mM Na_2EDTA and poured into gel-casting cassette (gel size, 120×250×1 mm). Samples were applied 2.5 cm from the cathodal end by the aid of an application foil and electrophoresed at 20 V/cm on a cooling block kept at 4°C until HbA marker migrated about 6 cm. BF bands were *in situ* precipitated by appropriately diluted polyclonal goat anti-BF serum (Atlantic antibodies, Scarborough, Me., U.S.A.).

Zymosan treatment of serum samples. Serum samples of BF S, BF FS, BF Fb1S, and BF F obtained in paternity cases were treated with zymosan A (Sigma, St. Louis, Mo., U.S.A.) at a ratio of 25 mg zymosan to 1 ml serum at 37°C for 15 hr (Raum *et al.*, 1984). Treated samples were subjected to same electrophoretic procedures described above.

Protein staining. Immunoprecipitated BF bands were visualized by usual Coomassie Brilliant Blue R-250 staining after washing overnight in saline.

C4 allotyping. C4 allotype was determined only for Yonaguni population according to the method described by Sim and Cross (1986). Cambodian samples

were not able to be typed owing to deterioration during sample collection and storage.

C2 allotyping. C2 allotype was determined by the technique described elsewhere (Suzuki *et al.*, 1986).

RESULTS AND DISCUSSION

Table 1 shows the distribution of BF phenotypes and allele frequencies in a Japanese population of Yonaguni island of Japan and a Cambodian population. Besides two common alleles, BF*F and BF*S, only BF*Fb1 was detected in a Japanese population of Yonaguni island but neither BF*Fb1 nor rare alleles were observed in a Cambodian population. Interestingly, BF*Fb1 occurred in a Japanese population of Yonaguni island at a frequency (0.0758) five times as high as in those of main islands of Japan where the frequency was reported to be 0.0154–0.0215 (Nakamura *et al.*, 1987; Suzuki *et al.*, 1987a; Nishimukai *et al.*, 1988). The observed numbers of each phenotype gave a good fit with the expected ones calculated by assuming Hardy-Weinberg equilibrium for each population. The electrophoretic patterns of native BF and of zymosan-treated BF are presented in Fig. 1. BF Fb1 migrated a little slower than BF F on agarose gel electrophoresis using TGVB. Electrophoretic variation of BF Fb1 resided in the Ba fragment, which migrated between that of BF F and BF S. Although we have no population data on the distribution of BF*Fb1, fortunately we found in disputed paternity cases a Korean family in which BF*Fb1 segregated from the accused man (not excluded) to one of three offsprings (Fig. 2). BF*Fb1 in this family was found to segregate together with C2*C, C4*A3, and C4B*2.

The BF allele frequencies among 19 Mongoloid populations were summarized in Table 2 with the data of this study. In Mongoloid populations, only two common

Table 1. Phenotypes distribution and allele frequencies.

	Cambodian		Japanese (Yonaguni island)	
	No. obs.	No. exp.	No. obs.	No. exp.
S	132	132.25	60	62.24
FS	42	41.49	24	20.61
F	3	3.25	1	1.71
Fb1S	0	0	13	11.90
Fb1	0	0	1	0.57
Total	177	176.99	99	97.03
BF*S	0.8644		0.7929	
BF*F	0.1356		0.1313	
BF*Fb1	0		0.0758	

$\chi^2=0.026$, 1d. f., $0.075<p<0.9$. $\chi^2=3.329$, 2d. f., $0.1<p<0.25$.

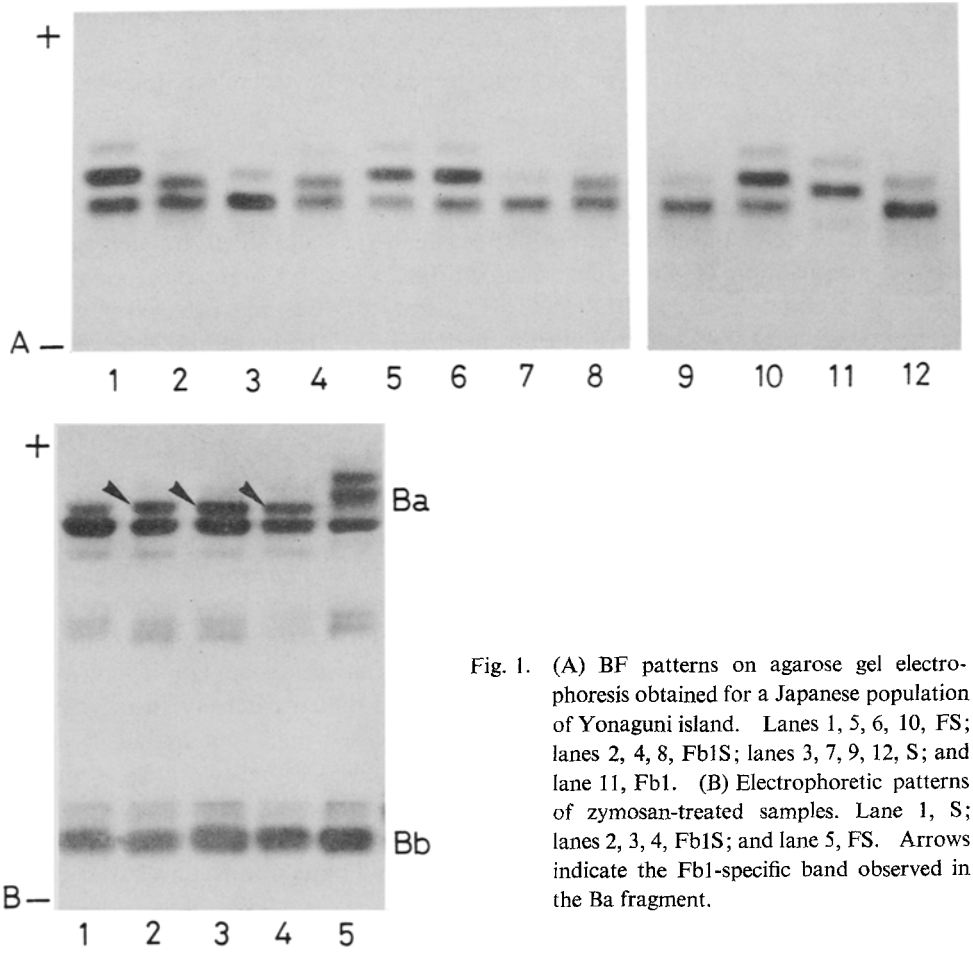

Fig. 1. (A) BF patterns on agarose gel electrophoresis obtained for a Japanese population of Yonaguni island. Lanes 1, 5, 6, 10, FS; lanes 2, 4, 8, Fb1S; lanes 3, 7, 9, 12, S; and lane 11, Fb1. (B) Electrophoretic patterns of zymosan-treated samples. Lane 1, S; lanes 2, 3, 4, Fb1S; and lane 5, FS. Arrows indicate the Fb1-specific band observed in the Ba fragment.

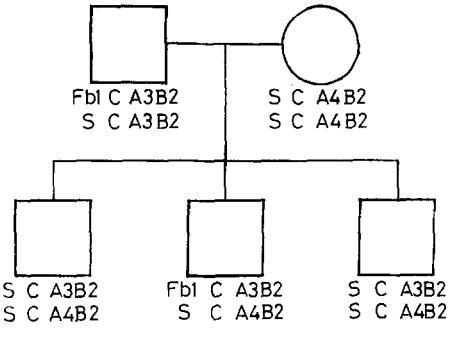

Fig. 2. Pedigree of a Korean family transmitting BF*Fb1. In this case the accused man was not excluded by using conventional polymorphic traits (7 blood groups, 11 blood cell enzymes, and 15 plasma proteins). Probability for paternity was estimated at 99.999%.

Table 2. Distribution of BF alleles among various Mongoloid populations.

Population	Residence	No	BF*F	BF*S	F/S	BF*RARE	BF*Fb1	Authors[c]
Chinese	Shanghai	200	0.1275	0.8700	0.15	S07	n.t.[b]	(1)
	Guangzhou	259	0.1197	0.8668	0.14	S07, SG1, FG2	n.t.	(2)
	Singapore	55	0.1000	0.9000	0.11	0	n.t.	(3)
	Bangkok	48	0.0833	0.8890	0.10	F025B, S03, S045	0.01	(4)
Korean	Seoul	220	0.2250	0.7750	0.29	0	n.t.	(5)
Filipinos	Manila	46	0.2830	0.7170	0.39	0	n.t.	(3)
	Manila	74	0.2973	0.7027	0.42	0	0	(4)
Thailander	Bangkok	184	0.0980	0.9020	0.11	0	n.t.	(6)
	Bangkok	45	0.1110	0.8890	0.12	0	n.t.	(3)
	Bangkok	72	0.1528	0.8472	0.18	0	0	(4)
Cambodian	n.i.[a]	177	0.1356	0.8644	0.16	0	0	(7)
Japanese	Nara	360	0.1760	0.8240	0.21	0	n.t.	(8)
	Tokyo	487	0.1980	0.8010	0.25	F075	n.t.	(9)
	Western Japan	534	0.1870	0.8120	0.23	F075	n.t.	(10)
	Tokyo	326	0.1825	0.7945	0.26	F075	0.0215	(11)
	Osaka	325	0.1492	0.8339	0.20	F075	0.0154	(12)
	Wakayama	53	0.1510	0.8400	0.18	F065	n.t.	(3)
	Western Japan	213	0.1408	0.8404	0.19	F075	0.0164	(13)
	Yonaguni	99	0.1313	0.7929	0.26	0	0.0758	(7)

[a] n.i., no information; [b] n.t., not tested; F+Fb1 was estimated for the calculation of F/S ratio. [c] (1) Zhao 1983, (2) Luo *et al.* 1987, (3) Miyano *et al.* 1986, (4) Suzuki *et al.* 1987b, (5) Park *et al.* 1985, (6) Greiner *et al.* 1980, (7) This study, (8) Horai 1976, (9) Tokunaga *et al.* 1982, (10) Nishimukai 1982, (11) Nakamura *et al.* 1987, (12) Suzuki *et al.* 1987a, (13) Nishimukai *et al.* 1988.

alleles, BF*F and BF*S, were detected with some other rare variants as compared with Caucasoid and Negloid populations in which two minor common alleles, BF*F1 and BF*S07, were observed in addition to the two common alleles. Although a little information is available on the distribution of BF*Fb1 among Mongoloid populations, BF*Fb1 occurs at least in Japanese, in Chinese and probably in Korean at polymorphic frequencies. BF*Fb1 is able to be detected by using agarose gel electrophoresis with TGVB in place of the standard barbital buffer for BF typing and by using IEF. IEF has been applied to BF subtyping, revealing the existence of two subtypes in the BF*F allele mainly in Caucasoid populations. We recently developed a new procedure for detecting the FA-FB subtype in the Ba fragment obtained after zymosan treatment (Suzuki *et al.*, 1989). Geserick *et al.* (1989) reported a Fb1-like variant named FB1 detected in a German family and later they have reported that this variant was different from BF*Fb1 in Mongoloid at the VIth Complement Genetics Workshop and Conference (Mainz, F.R.G., 1989). These data thus strongly indicated that BF*Fb1 may be characteristic of

some Mongoloid populations and is the third common allele at BF locus in Mongoloid like BF*F1 and BF*S07 in Caucasoid and Negloid.

When the data of Cambodian and Yonaguni were compared with those of various Mongoloid populations thus far studied (Table 2), the BF alleles distribution of Cambodian is very similar to those of its neighboring populations, Thai and Chinese in Singapore, in Thailand, and in mainland China (Shanghai and Guangzhou), but quite different from Filipinos despite their geographical proximity. In a Japanese population of Yonaguni, the increase of the BF*Fb1 frequency was accompanied to a larger extent by a decrease of the BF*F frequency than by a decrease of the BF*S frequency. This extremely high frequency of BF*Fb1 in the island people is ascribable to random genetic drift due to bottleneck effect. Among the Mongoloid populations listed in Table 2, the decrease of the BF*F frequency (the BF*Fb1 frequency was combined to the BF*F frequency for the populations marked with asterisk) and the increase of the BF*S frequency were reciprocally related to the north to south distribution of the populations concerned except for Filipinos of which the BF*F allele frequency were reversely related to their geographical location. This probable cline observed in the BF alleles distribution could be more prominently represented by taking the ratio of BF*F to BF*S. This ratio appeared to divide the populations into three groups, high value for Filipinos, medium value for Korean and Japanese, and low value for Chinese, Thai, and Cambodian. Much more data on other Mongoloid populations, however, must be obtained for further discussion.

Among numerous alleles at well-defined polymorphic loci, some are distributed exclusively in one of the three major races but are absent in the other two. As for Mongoloid populations, such specific alleles are typically illustrated by the Di^a antigen of the Diego blood group (Layrisse *et al.*, 1955), the Gmab3st haplotype of immunoglobulin heavy chain allotypes (Gm, for review see Matsumoto, 1988), the TFDChi allele of transferrin (for review see Kamboh and Ferrell, 1987), a set of rare mutant alleles of vitamin D binding protein (DBP, initially called group-specific component, Gc, for review see Constans *et al.*, 1985), and so on. The distribution of the rare variants such as TFDChi or DBP variants informs us anthropological relationship between populations, their exchanges, and their migrations (Constans *et al.*, 1985). Based on occurrence or absence of the BF*Fb1 allele, Mongoloids could be divided into two groups, which findings implied that two core populations might have occurred before the differentiation of Mongoloid into various groups. Such consideration is supported by the vast data on the two polymorphic loci, Gm and DBP. Analytical data on the Gm haplotype distribution among various Mongoloids provides an evidence for the occurrence of two distinct populations among paleo-Mongoloid populations of East Asia in the past (Matsumoto, 1988). In addition, Constans *et al.* (1985) deduced from the distributions of seven rare variants at DBP locus that considerable differentiation among Mongoloid populations existed, especially between northern and southern ones during the migration to the Americas.

Table 3. Association of Fb1 with other class III allotypes.

ID number	C4A	C4B	C2	BF
5	3, 3, 2	2, 0	C	Fb1S
8	3, 0	2, 1	C	Fb1S
17	4, 3	2, 2	C	Fb1S
31	3, 3	5, 2	C	Fb1S
37	3, 0	2, 1	C	Fb1S
39	3, 3	1, 1	C	Fb1S
43	4, 3	2, 2	C	Fb1S
44	3, 3, 2	1, 0	C	Fb1S
49	3, 0	2, 1	C	Fb1S
53	3, 3	2, 1	C	Fb1S
60	3, 3	2, 2	C	Fb1
90	3, 0	4, 2	C	Fb1S
91	3, 3	2, 1	C	Fb1S
99	3, 0	1, 0	C	Fb1S

	C4 A3 B2	
	(+)	(−)
Fb1	11	3
Others	17	68

$\chi^2 = 14.5$, $p = 0.00014$.

The BF locus is assigned between the locus for C2 and that for C4A within the MHC on chromosome 6 (Carroll *et al.*, 1984), which fact evokes further interest in the distribution of the Fb1 allele. As shown in Table 3, statistical analysis using 2×2 contingency table confirmed an extremely firm association of Fb1 phenotypes with C2*C and C4 phenotypes with C4A*3 and C4B*2, thus indicating a firmly linked haplotype, BF*Fb1, C2*C, C4A*3, and C4B*2 (Fb1C32). This result showed a good agreement with our previous study (Suzuki *et al.*, 1987a). Although only the other class III types were determined as a Fb1-associated complotype in this and the previous study (Suzuki *et al.*, 1987a), the location of the BF locus within the MHC region implies that the geographically limited distribution of the Fb1 allele might be related to the function of the other class I and class II alleles which are linked to the BF*Fb1 allele. Much better understanding of the Fb1 distribution will be provided by further investigations on northern Mongoloids populations with the information of the MHC class I and class II antigens.

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